

AMENDMENTS TO THE SPECIFICATION

Please make the following amendments to the specification.

Please replace paragraph 0023 with the following paragraph:

Reference is made to the appendix submitted herein. The appendix contains the following: SeqList_01.txt (24,689 KB), which was created January 26, 2007; SeqList_02.txt (24,680 KB); SeqList_03.txt (24,680 KB); SeqList_04.txt (24,680 KB); SeqList_05.txt (24,680 KB); SeqList_06.txt (24,680 KB); SeqList_07.txt (24,683 KB); SeqList_08.txt (24,680 KB); SeqList_09.txt (24,680 KB); SeqList_10.txt (24,680 KB); SeqList_11.txt (24,680 KB); SeqList_12.txt (24,680 KB); SeqList_13.txt (24,681 KB); SeqList_14.txt (24,680 KB); SeqList_15.txt (24,681 KB); SeqList_16.txt (24,680 KB); SeqList_17.txt (24,681 KB); SeqList_18.txt (24,681 KB); SeqList_19.txt (24,680 KB); SeqList_20.txt (24,680 KB); SeqList_21.txt (24,680 KB); SeqList_22.txt (24,680 KB); SeqList_23.txt (24,680 KB); SeqList_24.txt (24,680 KB); SeqList_25.txt (24,681 KB); SeqList_26.txt (24,680 KB); SeqList_27.txt (24,680 KB); SeqList_28.txt (24,680 KB); SeqList_29.txt (24,680 KB); SeqList_30.txt (24,680 KB); SeqList_31.txt (24,680 KB); SeqList_32.txt (24,680 KB); SeqList_33.txt (24,680 KB); SeqList_34.txt (24,680 KB); SeqList_35.txt (24,680 KB); SeqList_36.txt (24,680 KB); SeqList_37.txt (24,680 KB); SeqList_38.txt (24,680 KB); SeqList_39.txt (24,680 KB); SeqList_40.txt (24,680 KB); SeqList_41.txt (24,680 KB); SeqList_42.txt (24,698 KB); SeqList_43.txt (24,684 KB); SeqList_44.txt (24,680 KB); SeqList_45.txt (24,680 KB); SeqList_46.txt (24,680 KB); SeqList_47.txt (24,680 KB); SeqList_48.txt (24,680 KB); SeqList_49.txt (24,680 KB); SeqList_50.txt (24,680 KB); SeqList_51.txt (24,680 KB); SeqList_52.txt (24,680 KB); SeqList_53.txt (24,680 KB); SeqList_54.txt (24,680 KB); SeqList_55.txt (24,680 KB); SeqList_56.txt (24,680 KB); SeqList_57.txt (24,680 KB); SeqList_58.txt (24,680 KB); SeqList_59.txt (24,680 KB); SeqList_60.txt (24,680 KB); SeqList_61.txt (24,680 KB); and SeqList_62.txt (24,683 KB), which were created January 22, 2007; and SeqList_63.txt (9,135 KB), which was created January 26, 2007, which altogether are a sequence listing in accordance with 37 C.F.R. §§ 1.821-1.825, the contents of which are incorporated by reference herein.

Please replace paragraph 0263 with the following paragraph:

Fig. 23A is a schematic representation of an "operon-like" cluster of novel human hairpin sequences detected by a bioinformatic oligonucleotide detection engine constructed and operative in accordance with a preferred embodiment of the present invention, and non-GAM hairpin sequences used as negative controls thereto[;:]. The hairpins shown are as follows: N2 (SEQ ID NO: 10068286), N3 (SEQ ID NO: 10068287), MIR23 (SEQ ID NO: 10068288), GAM252 (SEQ ID NO: 10068289), GAM7617 (SEQ ID NO: 10068290), N252 (SEQ ID NO: 10068291), N4 (SEQ ID NO: 10068292), N0 (SEQ ID NO: 10068293), N6 (SEQ ID NO: 10068294), MIR24 (SEQ ID NO: 10068295), and N7 (SEQ ID NO: 10068296).

Please replace paragraph 0266 with the following paragraph:

Fig. 24A is an annotated sequence of EST72223 (SEQ ID NO: 10068281) comprising known human miRNA oligonucleotide MIR98 and novel human oligonucleotide GAM25 PRECURSOR detected by the oligonucleotide detection system of the present invention; ~~and~~. Additionally annotated in EST72223 are the miRNA-98 hairpin in bold (SEQ ID NO: 10068282), the sequence of the mature miRNA-98 in bold and underline (SEQ ID NO: 10068283), the sequence of the GAM25 hairpin in bold (SEQ ID NO: 10068284), and the sequence of the mature miRNA of GAM25 in bold and underline (SEQ ID NO: 10068285).

Please replace paragraph 0452 with the following paragraph:

The sequence presented in Row 29 is a representative of the group of five GAM RNAs. The full list of GAM RNA sequences and their corresponding precursors is as follows (each GAM RNA sequence is followed by the GAM Name): TCACTGCAACCTCCACCTCCCA (352092, 352651, 355761) (SEQ ID NO: 10068309), TCACTGCAACCTCCACCTCCCG (351868, 352440, 351973, 352169, 352445, 358164, 353737, 352382, 352235, 352232, 352268, 351919, 352473, 352444, 353638, 353004, 352925, 352943) (SEQ ID NO: 10068310), TCACTGCAACCTCCACCTCTG (358311) (SEQ ID NO: 10068311), TCACTGCAACCTCCACCTTCAG (353323) (SEQ ID NO: 10068312), and TCACTGCAACCTCCACCTTCCG (353856) (SEQ ID NO: 10068313).

Please replace paragraphs 0467-0469 with the following paragraphs:

Two types of cDNA libraries, designated "One-tailed" and "Ligation", were prepared from the one of the abovementioned fractionated RNA samples. RNA was dephosphorylated and ligated to an RNA (designated with lowercase letters)-DNA (designated with UPPERCASE letters) hybrid 5'-phosphorylated, 3'idT blocked 3'-adapter (5'-P-uuuAACCGCATCCTTCTC-idT-3' (SEQ ID NO: 10068314), Dharmacon #P-002045-01-05) (as elaborated in Elbashir et al., Genes Dev.15:188-200 (2001)) resulting in ligation only of RNase III type cleavage products.3'-Ligated RNA was excised and purified from a half 6%,half 13% polyacrylamide gel to remove excess adapter with a Nanosep 0.2 microM centrifugal device (Pall) according to instructions, and precipitated with glycogen and 3 volumes of ethanol. Pellet was resuspended in a minimal volume of water.

For the "Ligation" library, a DNA (UPPERCASE)-RNA (lowercase) hybrid 5'-adapter (5'-TACTAATACGACTCACTaaa-3' (SEQ ID NO: 10068315) Dharmacon #P-002046-01-05) was ligated to the 3'-adapted RNA, reverse transcribed with "EcoRI-RT": (5'-GACTAGCTGGAATTCAAGGATGCGGTAAA-3') (SEQ ID NO: 10068316), PCR-amplified with two external primers essentially as in Elbashir et al. (2001), except that primers were "EcoRI-RT" and "PstI Fwd" (5'-CAGCCAACGCTGCAGATACGACTCACTAAA-3') (SEQ ID NO: 10068317). This PCR product was used as a template for a second round of PCR with one hemispecific and one external primer or with two hemispecific primers.

For the "One-tailed" library, the 3'-adapted RNA was annealed to 20pmol primer "EcoRI RT" by heating to 70 C and cooling 0.1 C/sec to 30 C and then reverse-transcribed with Superscript II RT (according to manufacturer's instructions, Invitrogen) in a 20 microliters volume for 10 alternating 5 minute cycles of 37 C and 45 C. Subsequently, RNA was digested with 1 microliter 2M NaOH and 2mM EDTA at 65 C for 10 minutes. cDNA was loaded on a polyacrylamide gel, excised and gel-purified from excess primer as above (invisible, judged by primer run alongside) and resuspended in 13 microliters of water. Purified cDNA was then oligo-dC tailed with 400U of recombinant terminal transferase (Roche Molecular Biochemicals), 1 microliter 100 microM dCTP, 1 microliter 15mM CoCl₂, and 4 microliters reaction buffer, to a final volume of 20 microliters for 15 minutes at 37 C. Reaction was stopped with 2 microliters 0.2M EDTA and 15 microliters 3M NaOAc pH 5.2. Volume was adjusted to 150 microliters with water, Phenol:Bromochloropropane 10:1 extracted and subsequently

precipitated with glycogen and 3 volumes of ethanol. C-tailed cDNA was used as a template for PCR with the external primers "T3-PstBsg(G/I)18" (5'-AATTAACCCTCACTAAAGGCTGCAGGTGCAGGIGGGIIGGGIIGGGIIGN-3') (SEQ ID NO: 10068318) where I stands for Inosine and N for any of the 4 possible deoxynucleotides), and with "EcoRI Nested" (5'-GGAATTCAAGGATGCGGTTA-3') (SEQ ID NO: 10068319). This PCR product was used as a template for a second round of PCR with one hemispecific and one external primer or with two hemispecific primers.

Please replace paragraph 0471 with the following paragraph:

Hemispecific primers were constructed for each predicted GAM RNA oligonucleotide by an in-house program designed to choose about half of the 5' or 3' sequence of the GAM RNA corresponding to a TM of about 30-34C constrained by an optimized 3' clamp, appended to the cloning adapter sequence (for "One-tailed" libraries, 5'-GGNNGGGNNG (SEQ ID NO: 10068320) on the 5' end or TTTAACCGCATC-3' (SEQ ID NO: 10068321) on the 3' end of the GAM RNA; for "Ligation" libraries, the same 3' adapter and 5'-CGACTCACTAAA (SEQ ID NO: 10068322) on the 5' end of the GAM RNA). Consequently, a fully complementary primer of a TM higher than 60C was created covering only one half of the GAM RNA sequence permitting the unbiased elucidation by sequencing of the other half.

Please replace paragraph 0496 with the following paragraph:

Transcript products were 705 nt (EST72223), 102 nt (MIR98 precursor), 125 nt (GAM25 precursor) long. EST72223 was PCR amplified with T7-EST 72223 forward primer: 5'-TAATACGACTCACTATAGGCCCTTATTAGAGGATTCTGCT-3' (SEQ ID NO: 10068178) and T3-EST72223 reverse primer: 5'-AATTAACCCTCACTAAAGGTTTTTTTTTTCCTGAGACAGAGT-3' (SEQ ID NO: 10068179). MIR98 was PCR amplified using EST72223 as a template with T7MIR98 forward primer: 5'-TAATACGACTCACTATAGGGTGAGGTAGTAAGTTGTATTGTT-3' (SEQ ID NO: 10068180) and T3MIR98 reverse primer: 5'-AATTAACCCTCACTAAAGGGAAAGTAGTAAGTTGTATAGTT-3' (SEQ ID NO: 10068181). GAM25 was PCR amplified using EST72223 as a template with GAM25 forward primer: 5'-GAGGCAGGAGAATTGCTTGA-3' (SEQ ID NO: 10068182) and T3-EST72223 reverse primer: 5'-AATTAACCCTCACTAAAAGCCTGAGACAGAGTCTTGCTC-3' (SEQ ID NO: 10068183).

Please replace paragraph 0499 with the following paragraph:

Reference is now made to Fig.25A, which depicts a first method that uses primers designed to the stems of the hairpins. Since the stem of the hairpins often has bulges, mismatches, as well as G-T pairing, which is less significant in DNA than is G-U pairing in the original RNA hairpin, the primer pairs were engineered to have the lowest possible match to the other strand of the stem. Thus, the F-Stem primer, derived from the 5' stem region of the hairpin, was chosen to have minimal match to the 3' stem region of the same hairpin. Similarly, the R-stem primer, derived from the 3' region of the hairpin (reverse complementary to its sequence), was chosen to have minimal match to the 5' stem region of the same hairpin. The F-Stem primer was extended in its 5' sequence with the T3 primer (5'-ATTAACCCTCACTAAAGGGA-3' (SEQ ID NO: 10068184)) and the R-Stem primer was extended in its 5' sequence with the T7 primer (5'-TAATACGACTCACTATAGGG (SEQ ID NO: 10068185)). The extension is needed to obtain a large enough fragment for direct sequencing of the PCR product. Sequence data from the amplified hairpins is obtained in two ways. One way is the direct sequencing of the PCR products using the T3primer that matches the extension of the F-Stem primer. Another way is the cloning of the PCR products into a plasmid, followed by PCR screening of individual bacterial colonies using a primer specific to the plasmid vector and either the R-Loop (Fig. 25B) or the F-Loop (Fig. 25C) primer. Positive PCR products are then sent for direct sequencing using the vector-specific primer.

Please replace paragraphs 0559-0579 with the following paragraphs:

Sequence: 5'(5phos)rUrGrGCCTATAGTGAGTCGTATTA(3InvdT)3' (SEQ ID NO: 10068186)

2.Name:5Ada RNA-DNA XbaBseRI

Sequence:5'AAAGGAGGAGCTCTAGrArUrA 3' (SEQ ID NO: 10068187) or optionally:

3.Name:5Ada MC RNA-DNA PstAtaBser

Sequence:5'CCTAGGAGGAGGACGTCTGrCrArG 3' (SEQ ID NO: 10068188)

4.Name:3'Ada nT7 MC RNA-DNA

Sequence:5'(5phos)rCrCrUATAGTGAGTCGTATTATCT
(3InvdT)3' (SEQ ID NO: 10068189)

The following DNA primers are included in the present invention:

1.Name:T7 NcoI-RT-PCR primer

Sequence:5'TAATACGACTCACTATAGGCCA 3' (SEQ ID NO: 10068190)

2.Name:T7NheI SpeI-RT-PCR primer

Sequence:5'GCTAGCACTAGTTAATACGACTCACTATAGGC
CA 3' (SEQ ID NO: 10068191)

3.Name:5Ada XbaBseRI Fwd

Sequence:5'AAAGGAGGAGCTCTAGATA 3' (SEQ ID NO: 10068192)

4.Name:Pst-5Ada XbaBseRI Fwd

Sequence:5'TGACCTGCAGAAAGGAGGAGCTCTAGATA 3'
(SEQ ID NO: 10068193)

or optionally:

5.Name:5Ada MC PstAtaBser fwd

Sequence:5'ATCCTAGGAGGAGGACGTCTGCAG 3' (SEQ ID NO: 10068306)

6.Name:RT nT7 MC XbaI

Sequence:5'GCTCTAGGATAATACGACTCACTATAGG 3'
(SEQ ID NO: 10068307)

Please replace the paragraph in Table 10 that spans lines 738586-738597 with the following paragraph:

The GR12177 precursor encodes GR12177 precursor RNA, herein designated GR PRECURSOR RNA that is typically several hundred to several thousand nucleotides long. The nucleotide sequence of human GR12177 is located from position ~~58908413~~ 58901318 to 58908500 on the "+" strand of chromosome 19. The GR12177 precursor RNA folds spatially, forming the GR12177 folded precursor RNA, herein designated GR FOLDED

PRECURSOR RNA. It is appreciated that the GR12177 folded precursor RNA comprises a plurality of what is known in the art as hairpin structures. Hairpin structures result from the presence of segments of the nucleotide sequence of GR12177 precursor RNA in which the first half of each such segment has a nucleotide sequence which is at least a partial, and sometimes an accurate, reverse-complement sequence of the second half thereof, as is well known in the art.